Indices in differentiating iron deficiency anemia from thalassemia trait- a comparative study

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ABSTRACT

Background: Iron deficiency anemia (IDA) and beta thalassemia trait (BTT) are the two most common and important causes of microcytic hypochromic anemia in India. It is very difficult to differentiate between the two. Many different types of techniques have been proposed for the same. While some are invasive like bone marrow examination others are not available at all centers, like electrophoresis. Hence different indices come into play. **Aims and Objective:** This study was undertaken to compare the efficacy of Shine and Lal index and Mentzer index in differentiating between IDA and BTT. **Materials and Methods:** A total of 407 anemia cases were studied over a period of 18 months and their blood samples were subject to different hematological and biochemical assays to diagnose the type of anemia. **Results:** Based on these tests 92.1% cases were found to be of IDA whereas 3.7% cases were found to be of BTT. Then both the indices were applied in the above mentioned cases. **Conclusion:** While Shine and Lal index was found to have better sensitivity, Mentzer index was found to have better specificity.

Key words: Iron deficiency anemia; Beta thalassemia trait; Shine and Lal index; Mentzer index

INTRODUCTION

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It is the result of wide variety of causes that can be isolated, but more often co-exist. Globally, the most significant contributor to the onset of anemia is iron deficiency, so that Iron deficiency anemia (IDA) and Anemia are often used synonymously. It is generally assumed that 50% of the cases of anemia are due to Iron deficiency but the proportion may vary among population groups and in different geographical regions according to the local conditions.[1,2]

Morphologically, mirocytic anemias are among the most common type of anemias encountered by physicians and the two most common causes of microcytic anemia are IDA and Thalassemia trait (TT). IDA is characterized by pallor, fatigue and weakness. In severe IDA patient may present with koilonychia, alopecia, atrophic changes in the tongue & gastric mucosa. The diagnosis of IDA rests on laboratory studies. Peripheral blood smear shows microcytic hypochromic RBCs with anisopoikilocytosis. The Bone Marrow iron stores appear depleted. Hb, hematocrit, serum ferritin are reduced. Total Iron Binding Capacity is high.[2]

β-Thalassemia is an important disorder that has attracted the attention of medical research towards the multifaceted disease.[3-9] Most of the studies in the past have focused on β-thalassemia which is one of the most common single gene disorder with >4,00,000 newborns affected per year

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worldwide. [10] It is an autosomal hematological disorder resulting from genetically deficient synthesis of β-globin chain of Hb which leads to an excessive accumulation of α- globin chains. The extra α-globin chain precipitates and forms inclusion bodies in RBCs, which may be apparent in mildest β-Thalassemia Trait (BTT). The BTT patients are usually asymptomatic. They may present with mild anemia, headache or leg cramps. Peripheral blood smear shows same picture as IDA. [11]

It is important to differentiate between the IDA and BTT because each has entirely different etiology, pathogenesis, treatment and prognosis. Thus an unknown number of misdiagnosed thalassemia may be unnecessarily treated with iron. The real danger of misdiagnosed or non-diagnosis in carriers of BTT getting married, is a potential homozygous off spring.

In an era of rising cost consciousness, efficient diagnostic approaches which can rule in or out diseases with efficient accuracy so that testing is minimized, are welcome. Bone marrow studies are invasive methods and serum ferritin, serum transferrin and serum iron are relatively expensive tests, while Hb, RDW along with other red cell indices are part of routine testing in laboratories using automated hematology analyzers. If these easily available tests could be used to screen IDA with acceptably high sensitivity and specificity, the cost of anemia workup would drop considerably. Hence, keeping the above discussion in mind the present study was carried out to segregate cases of pure BTT from IDA.

In the past, various indices have been proposed to discriminate IDA from BTT according to geographical variations. These discrimination indices proposed by different workers are derived from different hematological parameters. [12]

In this study, we have applied two indices; Shine and Lal index and Mentzer Index in

discriminating Iron Deficiency Anemia from Thalassemia Trait. The Shine and Lal Index, described in 1977, mentioned that in their three years of study on hemoglobinopathies, HbA_2 was measured on 3734 samples, out of which 1973 cases were of microcytosis while 1761 were normocytic. The method of detecting and isolating BTT from IDA was carried out by applying the following newly derived formula based on red-blood cell indices.

Shine and Lal (S&L) Index = (MCV2 X MCH)/100

The values of less than <1530 was indicative of BTT and of more than >1530 was suggestive of IDA. [13]

Mentzer index is calculated from the results of a CBC. If the quotient of MCV (in fl) divided by the RBC (in millions/microlitre) is less than 13, thalassemia is said to be more likely. If the result is greater than 13, then IDA the possibility of IDA is higher. [14,15] It described in 1973 by Mentzer[16] and is said to be helpful in differentiating IDA from beta thalassemia. [17]

MATERIALS AND METHODS

The present study was carried out for a period of 18 months at a tertiary care hospital in North India. An informed and written consent from subjects was taken before enrolling them. Initially all the cases with microcytic hypochromic anemia attending medicine and pediatric wards and outpatient departments (OPD) were screened for inclusion and exclusion criteria. Thus 407 cases of microcytic hypochromic anemia were enrolled for study. The inclusion and exclusion criterion were as follows:

Inclusion criteria
Patients having blood pictures of mild microcytic hypochromic anemia.

Exclusion criteria
- Previously diagnosed cases of thalassemia or any hemolytic anemia.
- Well investigated case of IDA on iron therapy.
- Transfusion dependent thalassemia major.

All the samples were run on Sysmex 800X I cell counter and complete blood counts (CBC) were obtained, which included total and differential leucocyte counts, platelet parameters, red blood cell counts, hemoglobin, red cell distribution width, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). For microcytosis MCV < 80 and for hypochromia MCH <27 was taken as criterion.

After applying the discrimination indices, the suspected samples of IDA were confirmed by Serum ferritin (ELISA method), Serum Iron (Ferrozine method), TIBC (Ferrozine method) Those which were suspected to be BTT were confirmed through cellulose acetate hemoglobin electrophoresis at alkaline pH of 8.6 on cellulose acetate strips, at 450 V for 1 hour and 15 minutes.

Statistical analysis
The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 Statistical Analysis Software. The values were represented in Number (%) & Mean ± SD.

RESULTS

As previously discussed, a total of 407 clinically suspected subjects were enrolled in the study and were subjected to hematological assessment. Figure 1 shows the distribution of subjects according to outcome of hematological assessment.

Normal patients

Patients with normal iron profile as well as normal Hb electrophoretic pattern. Out of 407 subjects selected for the purpose of study, 375 (92.1%) had IDA, 15 (3.7%) were BTT, 5 (1.2%) were positive for thalassemia major, 1 (0.2%) for F-variant and 11 (2.7%) were normal.

Mean MCV, RDW, % RDW and S. TIBC levels of BTT patients was significantly lower as compared to IDA patients while mean hemoglobin level, S. Iron, S. Ferritin levels of BTT patients was significantly higher (Figure 2) as compared to that of IDA patients ($p<0.05$). For all the other parameters, statistically, no significant difference between two groups was observed ($p>0.05$)

Evaluation of Shine and Lal Index (Table 1) revealed the sensitivity, specificity, PPV, NPV, and accuracy to be 80%, 23.7%, 4%, 96.7% and 25.9% respectively. On calculating the performance of the diagnostic test using Youden'J statistic, it was found to be only 3.7%.

Evaluation of Mentzer Index (Table 2) revealed the sensitivity, specificity, PPV, NPV, and accuracy to be 46.7%, 97.6%, 43.8%, 97.8% & 95.6% respectively. On evaluating the performance of test by calculating Youden's J statistic, it was observed to be having a performance level of 44.3%.

DISCUSSION

The two most important causes of microcytic anemia are IDA and β-thalassemia trait.[16-18] Among the spectrum of β-thalassemia syndrome forms, BTT is the most common form encountered in India and is responsible for causing
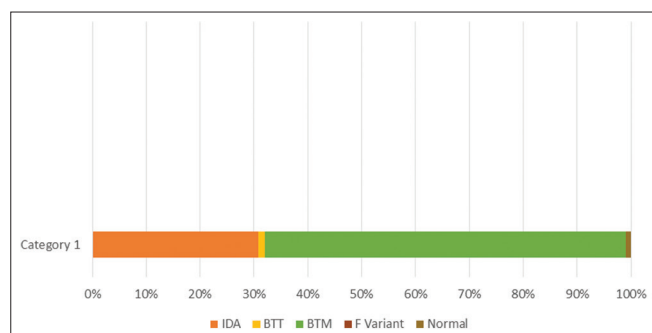
anemia other than IDA. However, a traditional approach followed by most general practitioners and blood bank physicians is a trial of iron treatment whenever anemia and/or microcytosis are encountered.[17] however this approach leads to unnecessary iron therapy/iron overload leading to oxidative stresses which leads to deterioration of patient instead of providing a cure. One of the reasons for adaptation of this approach is that burden of BTT is not exactly reported widely and at the same time the diagnosis of β-thalassemia is a cumbersome task which has both time as well as financial implications.[19-20]

In such a scenario, a hematologist is concerned about reducing the burden of cost and time in order to diagnose β-thalassemia. The present study is an attempt in this direction. Out of 407 subjects selected for the purpose of study, 375 (92.1%) had IDA, 15 (3.7%) were BTT positive, 5 were positive for thalassemia major, 1 (0.2%) for F-variant and 11 (2.7%) were normal. The mechanism by which anemia occurs alters the RBCs indices in a predictable manner. MCV is considered as the most informative parameter and anemias can be categorized as microcytic (MCV<78 fl), normocytic (MCV 80-90 fl), and macrocytic (MCV>100fl). Both β-thalassemia carriers and IDA cause microcytic anemia.[21]

In past several attempts have been made to develop a discrimination index which is suitable for discrimination between IDA and BTT. Some of these indices are – England and Fraser, [22] Mentzer,[14] Shine and Lal,[13] Srivastava,[23] Green and King,[24] Ricer et al.,[25] and Red Cell distribution Width (RDW) (Jayabose et al.,1999),[26] among many. In present study, we targeted two of these popular indices i.e., Shine and Lal and Mentzer indices for evaluation of their efficacy. The given table shows the efficacy of these indices evaluated in present study.

As shown in Table 3, between these two indices. Shine and Lal index (80%) is much more sensitive than Mentzer Index (46.7%). However, in terms of specificity Shine and Lal index is much less specific (23.7%) in comparison to

Figure 1: Distribution of patients according to outcome of hematological assessment

Table 1: Evaluation of sensitivity, specificity and efficiency of Shine and Lal index (n=407)			
Shine and Lal Index Values	**BTT**	**IDA**	**Total**
<1530	15[TP]	292[FP]	307
>1530	5[FN]	95[TN]	100
Total	20	387	407

Table 2: Evaluation of sensitivity, specificity and efficiency of Mentzer Index (n=407)			
Mentzer Index Values	**BTT**	**IDA**	**Total**
<13	7	9	16
>13	8	366	374

Mentzer Index (97.6%). Thus indicating that the burden of evaluation using screening process is quite high for Shine and Lal index. To attain the level of sensitivity and specificity as indicated by these indices, the overburden of false positivity was quite high ranging from 96% (Shine and Lal Index) to 56.2% (Mentzer Index).

Different studies from across the world have evaluated the performance of hematologic indices and found them to provide variable efficacy in different environments.

The findings in Table 4 show the sensitivity of Shine and Lal index ranging from 83.1% to 100% as compared to 80% and specificity ranged from 29.62% to 100% as compared to 23.7%. The positive predictive value of the test has been reported to be ranging from 41.8% to 100% in different series as compared to 4% whereas negative predictive value of the index has been reported to range from 3% to 100% in different series. The performance

of the index ranged from 28.87% to 100%. The poor performance of the index in present study might be attributed to very low prevalence of BTT positive patients in present study (3.8%). The maximum efficacy (100%) in a study by Higgins et al.,[27] might be attributed to high prevalence of BTT as well as the iron repleted status of the patients. In present study, no such replenishment was done. The findings in present study are close to that of Niazi et al.,[28] and Aziz et al.,[29] in terms of sensitivity, however, in terms of specificity they come close to the observations of Trivedi and Shah.[30] It must be kept in mind that prevalence rates affect the sensitivity, specificity, positive predictive value and negative predictive value in an influential manner.

The extreme variability in results with respect to performance of test indicates that Shine and Lal index has a limited utility which is dependent on prevalence as well as guided by time and place of study thus indicating that the empiricity of the index is questionable.

In present study, Mentzer index did not perform well as compared to various studies quoted above (Figure 3). Apart from the fact that the present study was carried out in a low prevalence scenario, this might also be due to the fact that we had taken the designated cut-off of <13 for discrimination. One of the studies by Higgins et al.,[27] also

Table 3: Efficacy of indices evaluated in present study

Index	Sensitivity	Specificity	PPV	NPV	Accuracy	Youden's Index
Shine and Lal	80	23.7	4	96.7	25.9	3.7
Mentzer Index	46.7	97.6	43.8	97.8	95.6	44.3

Table 4: Comparison of efficacy of Shine and Lal index in different studies

| Study (year)	Total No. (BTT)	Sensitivity	Specificity	PPV	NPV	Youden's Index
Sirdah et al. (1998)	262	97.2	49.7	41.8	97.9	46.9
Rathod et al. (2007)	200	94.8	70	-	-	64.8
Higgins et al. (2007)	327	100	100	100	100	100
Trivedi and Shah (2010)	216	99.25	29.62	88.88	44.8	28.87
Rahim and Keikhaei (2009)	323	100	89	82	100	89
Niazi et al (2010)	312	72	100	100	3	72
Aziz et al (2010)	901	83.1	90.6	89.6	86.4	73.7
Present Study (2013)	390	80	23.7	4	96.7	3.7
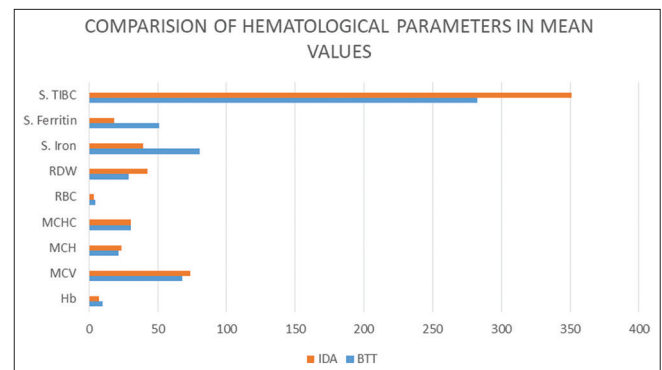
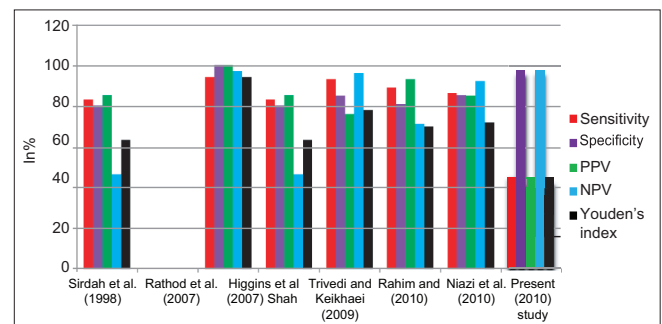**Figure 2:** Comparison of hematological parameters between IDA and BTT subjects

Figure 3: Shows a comparison of efficacy of Mentzer Index observed by different workers

used a modified prevalence scenario, relaxing the criteria for selection will have a very low impact on the specificity, however, it may increase the sensitivity substantially for this purpose. We performed a receiver-operator curve analysis to achieve a better cut-off which might have adequate sensitivity.

From the point of view of screening ability, the present study could not find any single index to be having adequate sensitivity as well as specificity, even the indices seeming to be promising carried a burden of high false positivity, thus rendering the purpose of screening to reduce the burden of overall final assessment and losing its practical use as a final test, though tests like Mentzer index were highly specific (97.6%) yet they had sensitivity level of 46.7% only.

Our study was peculiar from the point of view that it tested the efficacy of the test in a real life situation i.e., in a low prevalence setting, and the evaluation of the efficacy of different scales showed that the performance is grossly dependent on the prevalence scenario. The studies showing 100% performance of some indices are sample specific that have derived sample specific cut-off values which fail to stand the test of empiricity. However, the present study which was done in a real life simulation scenario had a high impact on the performance level owing to chance error. In present study, a chance false negative result affected the sensitivity by 6.7%, however in a purposively laid sampling design this error is reduced to as low as 0.6%. [27]

Despite being well accepted, the emergence of newer scales from time to time and testing-retesting of these indices shows that all the tests have their limitations which are felt during practical use but in a purposive sampling design these errors get suppressed and unrealistic performance levels of different tests are achieved.

Keeping in view the findings observed in present study, we would like to recommend testing of these indices for their empiricity by carrying out the performance of different indices in real-life situation rather than laying a biased purposive sampling design.

CONCLUSION

We hereby conclude that no single Discrimination Index can be applied to screen IDA from BTT, and application of multiple indices shall increase the sensitivity and specificity of the screening process. Our study was a prospective one and of a short duration yielding smaller number of BTT cases in a low prevalence zone of thalassemia. Further studies of sensitivity and specificity of many discrimination indices on a larger sample may yield some conclusive results.

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